Interaction of Soy Isolate with Polysaccharide and Its Effect on Film Properties

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When soy isolate was mixed with sodium alginate, the two polymers interacted to form electrostatic complexes. They also formed varying degrees of covalent bonding, depending on reaction time and the presence or absence of the reducing agent sodium cyanoborohydride. On the other hand, soy isolate and propyleneglycol alginate (PGA) formed mostly covalent complexes at alkaline pH. The interaction of soy protein with polysaccharide maintained or improved its solubility and emulsifying activity, particularly when covalent bonds were involved. The alkylated complexes also showed better film-making properties. However, protein–PGA films were more readily formed and had greater stability in water than the protein–alginate films.

KEY WORDS: Alkylation, elongation, films, glycosylation, protein, protein functionality, soy isolate, tensile strength.

Proteins and polysaccharides are polymers, each with its own unique physicochemical properties. Recently there has been considerable interest in the interaction of these two polymers to develop functional properties for use in food and nonfood products. Acidic polysaccharides, such as alginate, pectate and carboxymethyl cellulose, are known to form charge–charge electrostatic complexes with protein (1). Thus, alginate has been used to separate protein from sources such as process water, and the resulting protein–alginate complex was prepared into a range of proteinaceous foams, gels and other value-added products (2). Similarly, interactions between polysaccharide (gum arabic and arabinogalactan) and lipid–protein complexes have been utilized to remove lipids from yeast protein (3).

Interactions that form covalent bonds between protein and polysaccharide are particularly desirable, because the bonded complex will be more stable to heat, ionic effects, and other conditions. A soluble conjugate of ovalalbumin and dextran, covalently crosslinked *via* the naturally occurring Maillard reaction, has been prepared by heating a mixture of the components in a moisture chamber (4). The product exhibited excellent emulsifying properties and heat stability (5). Covalent bonding between protein and propyleneglycol alginate (PGA) has been extensively investigated (6,7). Gelatin–PGA gels showed unique hardening properties, which were utilized to prevent melting, as well as to control swelling during photographic developing processes (8).

Direct coupling of sugar or polysaccharide to protein has also been achieved by reductive alkylation with sodium cyanoborohydride as the reducing agent (9,10). The method has been used to glycosylate the 11S storage pea protein (11–13) and casein (14). Generally, functional properties for the glycosylated protein, such as emulsifying activity, were improved, but nutritional values were slightly decreased.

In this report, interactions of soy isolate and polysaccharides (alginate and PGA) were investigated. The protein was alkylated with sodium alginate in the presence and absence of cyanoborohydride. It was also treated with PGA under various pH conditions. Effects of the interaction on film-making and other functional properties were evaluated.

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MATERIALS AND METHODS

Chemicals. Soy protein isolate (Purina 620) with 90% protein and 80% nitrogen solubility index (NSI) was provided by Ralston Purina Co. (St. Louis, MO), and soy protein hydrolysate (D100WA) with 78% protein and 98% NSI was provided by A.E. Staley Mfg. Co. (Decatur, IL). Sodium alginate (Kelgin LV) and PGA (Kelcoloid S) were obtained from the Kelco Division of Merck & Co. (Rahway, NJ). The protein assay reagent bicinchoninic acid (BCA) was from Pierce Chemical Co. (Rockford, IL), sodium cyanoborohydride from Aldrich Chemical Co. (Milwaukee, WI) and *o*-phthalaldehyde (OPA) from Sigma Chemical Co. (St. Louis, MO). All other reagents and chemicals used were of ACS reagent grade.

Reductive alkylation. A dispersion was prepared by mixing 1 g each of soy isolate and sodium alginate in 100 mL of 0.15 M sodium bicarbonate buffer (pH 8.5). Sodium cyanoborohydride (0.3 g) was added to the mixture and the reaction was carried out by stirring at 37°C for 70 h. The product was dialyzed for 72 h against three changes of water at 4°C, and was then freeze-dried.

Protein–PGA interaction. A solution was prepared by dissolving 1.3 g PGA in 200 mL water. Two grams of soy isolate was added to the solution, and the pH of the mixture was adjusted to 6 or 9 by HCl or NaOH. The reaction was carried out at room temperature for 30 min. At the end of the reaction, the pH of the mixture was adjusted to 6, and the product was recovered by lyophilization.

Protein–alginate films. Protein–alginate films were prepared as follows. A solution was prepared by dissolving 5 g sodium alginate in 300 mL water. Forty g of the solution was added to a mixture containing 3 g water, 3 g soy isolate and 2 g glycerin. The resulting slurry was homogenized and then cast on a glass plate with a film spreader. A 3-mm spreader clearance was used to achieve the desired film thickness of 5–9 mils. For films with reductive alkylation, 0.4 g sodium cyanoborohydride was added to the slurry prior to the mixing and casting. After drying in a 50°C oven for 10–15 min, films both with and without sodium cyanoborohydride were incubated in a chamber with 85% RH (relative humidity under saturated KCl) at 50°C for 3 wk. At the end of incubation, the films were removed from the plate and conditioned at 65% RH for 48 h before analysis.

Protein–PGA films. Protein–PGA films were prepared as follows: A solution was prepared by dissolving 2.0 g PGA in 150 mL water. Twenty g of the solution was added to a mixture containing 3 g water, 3 g soy isolate and 2 g glycerin. The resulting slurry was homogenized and then cast on a glass plate with a film spreader at 1-mm clearance. A smaller spreader clearance was used here than the one for protein–alginate films to produce the desired

film thickness of 5–9 mils. After drying in an oven at 50°C for 10–15 min, the film was treated with 0.5 M phosphate buffer (pH 8) or 0.5 M KOH by the same casting method. The film was dried as before, followed by a casting treatment with 5% acetic acid. After final drying, the film was removed from the glass plate and stored in a chamber with 65% RH before analysis.

Protein analysis. Sodium decylsulfate polyacrylamide gel electrophoresis (4–20% gradient) was performed according to Laemmli (15). Size-exclusion chromatography (SEC) analysis was conducted on a Waters 600E liquid chromatograph with a Waters 1040 detector (Millipore Corp., Bedford, MA) and a Pharmacia Superose 12 gel column (Pharmacia Biotech Inc., Piscataway, NJ). Sodium phosphate buffer (50 mM, pH 7.0, 150 mM NaCl) was used as eluant, and the elution was monitored at 280 nm. Protein content was determined by the BCA method (16), and free amino groups in protein by the OPA assay according to Church *et al.* (17).

Functionality analysis. Turbidity at 600 nm was determined on 1% (wt/vol) dispersion of the protein–polysaccharide sample in distilled water adjusted to pHs ranging from 2.0 to 8.5. Emulsification activity index (EAI), expressed as interfacial area/unit weight protein (m^2/g), was assessed by the turbidometric method of Pearce and Kinsella (18).

Film analysis. Tensile strength and elongation of the films were analyzed (ASTM D882-83) with an Instron Universal Testing Machine (Model 4201; Instron Engineering Corp., Canton, MA). Films were preconditioned at 21.1°C and 65% RH for 48 h before analysis (a modification from the 25°C and 50% RH normally used). Film thickness was determined as an average of five measurements with a dial micrometer. The tensile strength in kilopascal (kPa) was calculated by dividing the maximum load by the cross-sectional area of the film. The percentage elongation was calculated from the maximum elongation during testing vs. the original gauge length of the film. Water vapor mass flow rate was recorded as moisture gain times film thickness vs. time by the cup method (ASTM E96-80; Ref. 19) in Carson dishes (No. 305-8; Thwing-Albert, Philadelphia, PA) with anhydrous calcium chloride as desiccant.

RESULTS AND DISCUSSION

Protein–alginate interaction. When soy protein isolate was mixed with sodium alginate in water in the presence of sodium cyanoborohydride, interaction between protein and polysaccharide was confirmed by polyacrylamide gel electrophoresis analysis. Sharp electrophoretic bands, representing the protein subunits, became less well defined, new bands emerged, and some old ones disappeared (Fig. 1). However, key bands in the profile remained, indicating incomplete reaction. According to OPA analysis, about 11.3% of the free amino groups were alkylated.

Effect of protein–alginate interaction. The partially alkylated protein products displayed substantial changes in their functional properties. Figure 2 shows turbidity profiles of the samples as functions of pH. A typical bell-shaped profile was observed for the untreated soy protein. With the addition of alginate but without covalent alkylation, turbidity of the mixture decreased and showed a deformed bell-shaped profile. Electrostatic charge–charge

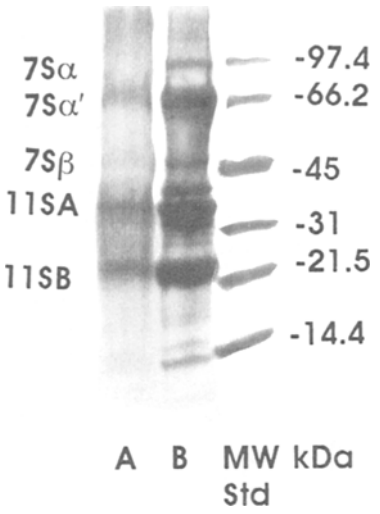

FIG. 1. Electrophoretic profiles for (A) soy isolate treated with alginate in the presence of sodium cyanoborohydride and (B) intact soy isolate.

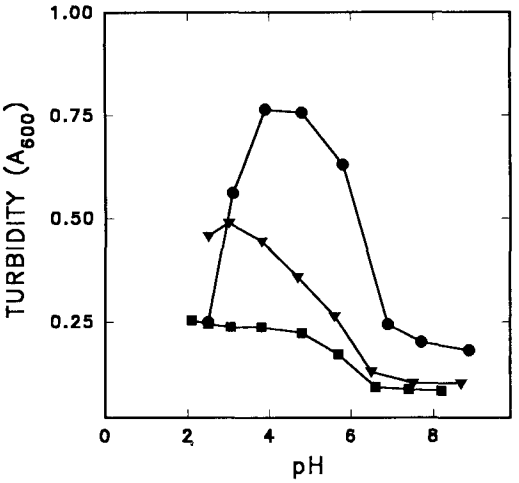

FIG. 2. Turbidity profiles of soy proteins as functions of pH. Intact soy protein (●); soy protein interacted with sodium alginate in the absence of sodium cyanoborohydride (▼); and soy protein interacted with sodium alginate in the presence of sodium cyanoborohydride (■).

interactions between the protein and polysaccharide are believed to be responsible for this change in solubility (1). The sample produced in the presence of the reducing agent gave an almost flat profile at still lower turbidity level, indicating improvements in solubility over a wide range of pH. Apparently, interaction of soy protein with sodium alginate, either covalent or electrostatic, resulted in enhanced hydration and improved solubility.

On the other hand, effects on emulsifying activity were evident only for samples with some covalent bonds between the protein and alginate components. EAI profiles of the samples as functions of pH (Fig. 3) show that soy protein in the absence or in the presence of alginate did a poor job of emulsifying oil. However, substantial improvement was observed for the alkylated protein product.

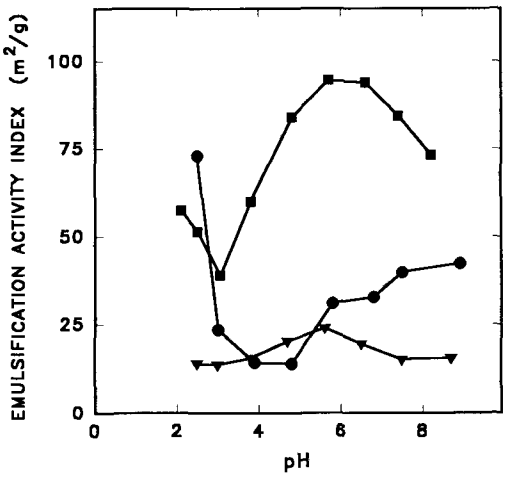

FIG. 3. Emulsifying activity indices of soy proteins as functions of pH. Intact soy protein (●); soy protein interacted with sodium alginate in the absence of sodium cyanoborohydride (▼); and soy protein interacted with sodium alginate in the presence of sodium cyanoborohydride (■).

Protein-PGA interaction. When soy protein isolate or soy protein hydrolysate was treated with PGA, the extent of interaction varied depending on pH. Little reaction was observed below pH 6, and it increased significantly with increased pH above pH 8. Figure 4 shows the SEC chromatograms during the reaction of PGA with soy protein hydrolysate at pH 9. As covalent coupling increased with time, high molecular weight species, which eluted immediately after the void volume, increased during SEC analysis.

Effects of protein-PGA interaction. Figure 5 shows the difference between turbidity profiles with and without protein-PGA alkylation. Compared with the profile of the protein control, the profile from treatment with PGA at pH 6 had similar heights but broader shoulders. The results indicate that, on the addition of PGA, but with limited covalent coupling, the solubility of the protein decreased in a narrow range of pH near the isoelectric point. With increased covalent coupling at pH 9, the turbidity profile shifted to the left to lower pH with relatively lower maximum turbidity. Solubility appeared to increase at higher levels of protein-PGA interaction, but the improvement remained relatively small. The effect of the interaction on emulsion activity is shown in Figure 6. The profiles demonstrated substantial increases in emulsion activity at pH 4 and above for the protein-PGA samples, particularly for the one prepared at pH 9.

Protein-alginate films. Mixtures of soy isolate and sodium alginate were investigated for their abilities to make films. In the absence of the reductive alkylating agent sodium cyanoborohydride, the film formed was brownish in color, indicating Maillard reaction. The Maillard reaction not only caused the color but also cross-links the two components through natural alkylation (4). In the presence of sodium cyanoborohydride, reductive alkylation instead of the normal Maillard reaction occurred, and a lighter color and more transparent film were formed. Films readily removed from the glass plate could be obtained at alginate-to-protein ratios of 1:5 (w/w) and

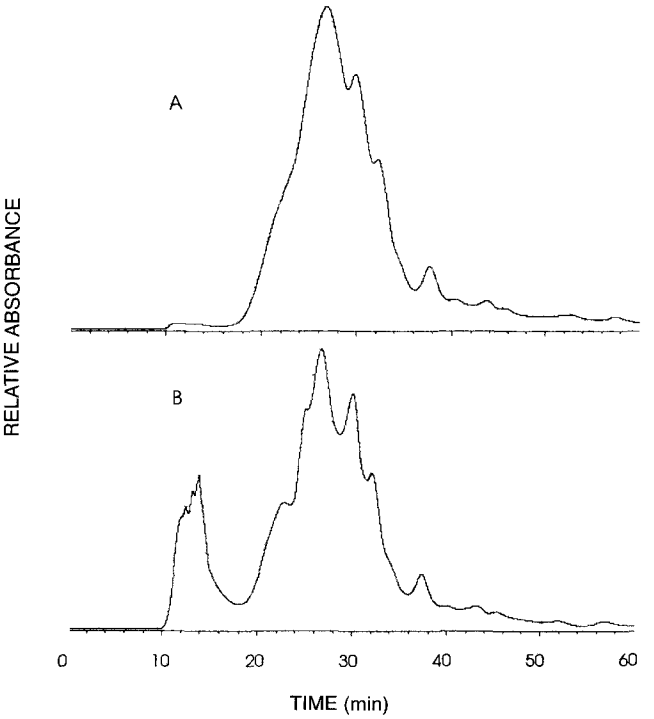

FIG. 4. Size-exclusion chromatograms of soy protein hydrolysates with propyleneglycol-alginate at pH 9 at (A) 0 h and (B) 2 h.

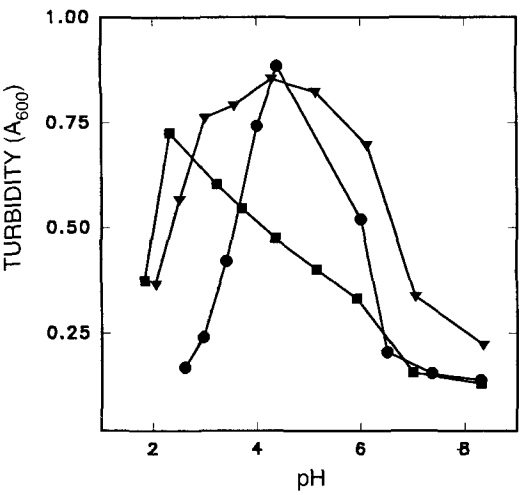

FIG. 5. Turbidity profiles of soy proteins as functions of pH. Intact soy protein (●); soy protein interacted with propyleneglycol-alginate (PGA) at pH 6 (▼); and soy protein interacted with PGA at pH 9 (■).

higher. However, the rate of alkylation was slow, particularly for the natural alkylation *via* Maillard reaction. After a three-week incubation, free amino groups decreased (or alkylation increased) by 36.1 and 10.8% for films alkylated with and without sodium cyanoborohydride, respectively. Apparently, formation of protein-alginate films by alkylation, with and without sodium cyanoborohydride, was slow and ineffective.

Protein-PGA films. When an aqueous slurry of soy protein and PGA was used, the resulting film varied depend-

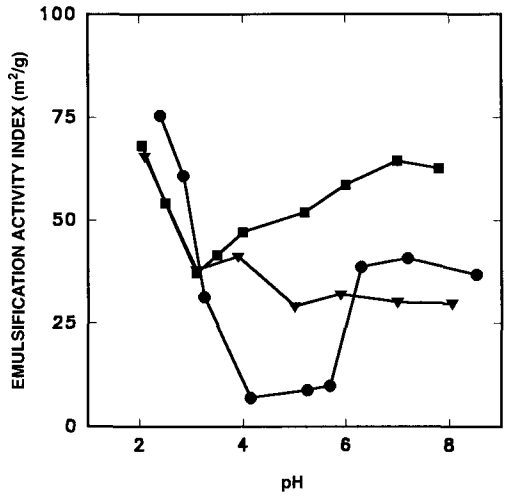

FIG. 6. Emulsifying activity indices of soy proteins as functions of pH. Intact soy protein (●); soy protein interacted with PGA at pH 6 (▼); and soy protein interacted with PGA at pH 9 (■). Abbreviation as in Figure 5.

ing on pH. Stronger and more tenacious films formed under alkaline conditions when the protein and PGA reacted to form covalent bonds. The crosslinking was caused mostly by the amidation between ε-amino groups of lysine residues in the protein and manuronic acid esters of the polysaccharide (6). Little natural alkylation, as described previously, occurred because of the short time involved. A good film, which could be easily peeled off the glass, resulted if the mixture was allowed to react at pH 8 and higher. Relatively low levels of PGA were needed, and good-quality films were obtained at ratios of PGA to protein as low as 1:10 (w/w). At pH 8 and higher, about 24.4% of the amino groups in the protein were covalently bonded to PGA at 50°C in less than 30 min. The product was quickly neutralized to prevent the remaining PGA from being hydrolyzed. Both soy isolate and PGA are safe for human consumption and, under the mild conditions used, no undesirable by-products are generated during the interaction. Therefore, the resulting film should be edible.

Film properties. Films were soaked in water or in 100 mM NaCl solution to test their stability and integrity. The protein-alginate films, prepared with or without sodium cyanoborohydride, swelled extensively and disintegrated when stirred in water. After 6 h, about 6–9% of the protein was released into the extracting solution either with or without salt. Under similar soaking conditions, the protein-PGA films maintained their forms with little swelling. The extracted protein contents were 4.1 and 6.7% for films prepared with 0.5 M KOH and pH 8 buffer, respectively. The type of polysaccharide and the extent of covalent bonding appear to be the determining factors for the film's stability in water.

Tensile strength and percentage elongation of films are listed in Table 1. For reductive alkylation, the films with and without sodium cyanoborohydride had tensile strengths of 1034 and 821 kPa, respectively. Their respective elongations were 30.5 and 15.5%. The film with sodium cyanoborohydride was more elastic and could be easily recovered from the glass plate. Apparently, cyanoborohydride-initiated alkylation was more effective than the natural alkylation process in producing protein-alginate films.

For the protein-PGA films, tensile strengths were in the range of 779–1158 kPa, and elongation ranged from 18–23%. The improvements from treatment with pH 8 buffer to 0.5 M KOH were relatively insignificant. On the other hand, films treated with 0.5 M KOH had better resistance to water vapor than those treated with pH 8 buffer (Fig. 7). Modification of the KOH-treated film, by addition of a small amount of lauric acid to the casting slurry, did not change its tensile strength or percentage elongation, but substantially increased its water vapor resistance (Fig. 7). Incorporation of water-repelling additives appears achievable and beneficial for the protein-PGA films.

This study demonstrates, with soy isolate, sodium alginate and PGA as models, that interactions between protein and polysaccharide, particularly when covalent bonding is involved, enhance film-forming properties. However, the film-forming interaction *via* natural Maillard reaction takes too long to be practical. The one induced by reductive alkylation, though relatively more effective, requires a specific reducing agent, sodium cyanoborohydride, which is unsuitable for food uses. On the other hand, the PGA alkylation process seems promising. Because PGA is not only safe for human consumption but also highly effective and efficient in alkylating proteins, it has the potential to be used in the preparation of protein-based edible films.

TABLE 1

Tensile Strength and Percent Elongation for Films Prepared by the Interaction of Soy Isolate with Sodium Alginate or PGA[a]

Soy protein films	Thickness (mils)	Tensile strength (kPa)	Elongation (%)
With sodium alginate in the absence of cyanoborohydride	6.1	821 ± 90	15.5 ± 0.9
With sodium alginate in the presence of cyanoborohydride	7.0	1034 ± 124	30.5 ± 5.1
With PGA and pH 8	5.5	779 ± 76	18.3 ± 0.9
With PGA and KOH	5.8	848 ± 69	22.8 ± 1.5
With PGA, KOH and lauric acid	8.6	1158 ± 138	20.5 ± 6.0

[a]Thickness was the mean of five measurements. Tensile strength and elongation were the mean of three determinations ± one standard deviation. PGA, propyleneglycol alginate; kPa, kilopascal.

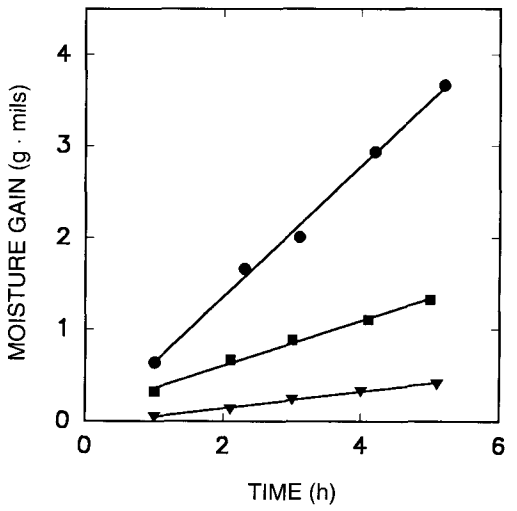

FIG. 7. Moisture gain as a function of time for films prepared by the interaction of soy isolate and PGA. Protein–PGA films prepared with buffer at pH 8 (■); prepared with dilute KOH (●); and prepared with dilute KOH and the addition of lauric acid (▼). Abbreviation as in Figure 5.

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